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Study on the relationship between the methylation of the MMP-9 gene promoter region and diabetic nephropathy

Badanie zależności między metylacją regionu promotora genu MMP-9 a nefropatią cukrzycową

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Abstract

Introduction: This study aims to explore the relationship between the methylation of matrix metalloproteinase (MMP)-9 gene promoter region and diabetic nephropathy (DN) through the detection of the methylation level of MMP-9 gene promoter region in the peripheral blood of patients with DN in different periods and serum MMP-9 concentration.

Material and methods: The methylation level of the MMP-9 gene promoter region was detected by methylation-specific polymerase chain reaction (MSP), and the content of MMP-9 in serum was determined by enzyme-linked immunosorbent assay (ELISA).

Results: Results of the statistical analysis revealed that serum MMP-9 protein expression levels gradually increased in patients in the simple diabetic group, early diabetic nephropathy group, and clinical diabetic nephropathy group, compared with the control group, and the difference was statistically significant ($P < 0.05$). Compared with the control group, the methylation levels of MMP-9 gene promoter regions gradually decreased in patients in the simple diabetic group, early diabetic nephropathy group, and clinical diabetic nephropathy group, and the difference was statistically significant ($P < 0.05$). Furthermore, correlation analysis results indicated that the demethylation levels of the MMP-9 gene promoter region were positively correlated with serum protein levels, urinary albumin-to-creatinine ratio (UACR), urea, and creatinine, and was negatively correlated with GFR.

Conclusions: The demethylation of the MMP-9 gene promoter region may be involved in the occurrence and development of diabetic nephropathy by regulating the expression of MMP-9 protein in serum. **(Endokrynol Pol 2018; 69 (3): 269–275)**

Key words: *diabetic nephropathy; matrix metalloproteinase-9; DNAmethylation*

Streszczenie

Wstęp: Celem pracy jest zbadanie zależności między metylacją regionu promotora genu metaloproteinazy macierzy zewnątrzkomórkowej typu 9 a nefropatią cukrzycową, poprzez wykrycie poziomu metylacji regionu promotora genu MMP-9 we krwi obwodowej pacjentów z nefropatią cukrzycową w różnych okresach i przy różnym stężeniu MMP-9 w surowicy krwi.

Materiał i metody: Poziom metylacji regionu promotora genu MMP-9 wykrywano za pomocą metylospecyficznej reakcji łańcuchowej polimerazy (*methylation-specific polymerase chain reaction*; MSP), natomiast zawartość MMP-9 w surowicy krwi była określana przy użyciu enzymatycznego testu immunoadsorpcyjnego (ELISA).

Wyniki: Wyniki analizy statystycznej wykazały, że poziom ekspresji białka MMP-9 w surowicy krwi stopniowo wzrastał w grupie pacjentów ze zwykłą cukrzycą, w grupie pacjentów z wczesną nefropatią cukrzycową oraz w grupie z kliniczną nefropatią cukrzycową w porównaniu z grupą kontrolną; różnica była statystycznie istotna ($p < 0,05$). W porównaniu z grupą kontrolną poziom metylacji regionów promotora genu MMP-9 stopniowo się zmniejszał w grupie pacjentów ze zwykłą cukrzycą, w grupie pacjentów z wczesną nefropatią cukrzycową oraz w grupie z kliniczną nefropatią cukrzycową; różnica była istotna statystycznie ($p < 0,05$). Ponadto, wyniki analizy korelacji wykazały, że poziomy demetylacji regionu promotora genu MMP-9 były dodatnio skorelowane ze stężeniem białek w surowicy krwi, ze wskaźnikiem albumina/kreatynina (*urinary albumin to creatinine ratio*; UACR), mocznikiem i kreatyniną oraz były ujemnie skorelowane ze wskaźnikiem GFR.

Wnioski: Demetylacja regionu promotora genu MMP-9 może być zaangażowana w występowanie i rozwój nefropatii cukrzycowej poprzez regulację ekspresji białka MMP-9 w surowicy krwi. **(Endokrynol Pol 2018; 69 (3): 269–275)**

Słowa kluczowe: *nefropatia cukrzycowa, metaloproteinaza macierzy zewnątrzkomórkowej typu 9, metylacja DNA*

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Introduction

Diabetic nephropathy (DN) is a complex polygenic disease that involves many pathogenic genes, and is caused by the accumulated interactions of genetic and environmental factors. It is of great significance for exploration of the pathogenesis, early diagnosis, prevention and delay occurrence and development of diabetic nephropathy, in order to improve the survival rate of diabetic patients and their quality of life. Matrix metalloproteinases (MMPs) are a group of zinc-dependent endopeptidases with common biochemical properties that are closely related to normal physiological and abnormal pathological changes of the kidney [1, 2]. Experiments in vitro have confirmed that high concentrations of glucose and advanced glycation end-products can affect the expression level of MMP-9 in renal parenchymal cells. It was found in the cultivation of lymphocytes that the mRNA and protein expression levels of MMP-9 were negatively correlated with the methylation status of the MMP-9 gene promoter [3]. Roach et al. [4] found in studies that the high expression of MMP-9 was associated with the low methylation level of the MMP-9 gene. The demethylation of the MMP-9 gene promoter may play an important role in the occurrence and development of tumours by enhancing the mRNA expression of MMP-9 and promoting the secretion of MMP-9 protein.

At present, there are no related local or foreign reports that confirm the correlation between the methylation regulation of the MMP-9 gene promoter and DN, the difference in methylation status of the MMP-9 gene promoter in patients in different DN stages, and the involvement of the MMP-9 gene promoter in the pathogenesis of DN. In order to further explore the role of the methylation status of the MMP-9 gene promoter in the pathogenesis of DN and the possible epigenetic regulation mechanism, patients with DN were selected as the research objects, their MMP-9 levels were detected, and the methylation status of the MMP-9 gene promoter was detected by methylation-specific PCR. All these were carried out to elucidate the role of the methylation status of the MMP-9 gene promoter in the pathogenesis of DN, provide a new theoretical basis for the occurrence and development of DN, and provide new ideas for the early prevention and treatment of DN.

Material and methods

Experimental methods

Research objects

The research objects were patients who continuously visited the Outpatient Endocrinology Department of the Affiliated Zhongshan Hospital of Dalian University from January 2015 to December 2015. These patients were diagnosed with impaired glucose regulation, simple type-2 diabetes mellitus, and early and clinical stage of DN; and each included 30 cases. Patients who suffered from infection disease, high blood pressure, malignant tumour, coronary heart disease, heart failure, and obesity, and patients who were administrated with renal toxic drugs were excluded. Impaired glucose regulation and diabetes mellitus were diagnosed according to the criteria for diagnosis and classification of diabetes mellitus (WHO 1999). DN was divided into two categories by ACR, according to the Chinese type-2 diabetes prevention and treatment guidelines (2013 Edition): early diabetes (ACR: 2.5––30.0 mg/mmol [male], 3.5–30.0 mg/mmol [female]) and clinical diabetes mellitus (ACR > 30.0 mg/mmol). All the patients with diabetic nephropathy simultaneously underwent a fundus examination to confirm whether they were combined with diabetic retinopathy. In addition, another 30 cases of healthy volunteers who took physical examinations at the same time were selected as the control group. All subjects were informed and provided signed informed consent. This study was approved by the Ethics Committee of the Hospital.

Research methods

Specimen collection

1. General data collection: Data collected from all subjects included gender, age, duration of the disease, body mass index (BMI), fasting plasma glucose (FPG), glycosylated haemoglobin (HbA_{1c}), systolic blood pressure (SBP), diastolic blood pressure (DBP), triglyceride (TG), total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C), blood urine nitrogen (BUN), creatinine (CR), urinary albumin/creatinine (UACR), and glomerular filtration rate (GFR).
2. Five millitres samples of elbow venous blood were collected from all subjects 8–12 hours after fasting. Two copies were collected per specimen. One copy was placed in a non-anticoagulant tube and shaken. The serum was separated within four hours and centrifuged at 3500 rpm for 10 minutes. Then, the serum in the upper layer was extracted and stored in a refrigerator at –80°C to detect the concentration of MMP-9 (within two months). The other copy was placed in an EDTA anticoagulation tube at –80°C for application (within two months).

Detection of methylation status of the MMP-9 gene

1. **Specimen DNA extraction:** The genomic DNA was extracted using an Ezup column blood genomic DNA extraction Kit (Shanghai Shenggong Biological Engineering Co., Ltd.). Operations were performed strictly in accordance with the operation instructions, based on the requirement.

2. **DNA modification by bisulphite transformation:** The extracted leukocyte DNA was modified by bisulphite sodium, in strict accordance with the instructions of the kit, and the unmethylated cytosine (C) in the sequence was transformed into uracil (U). The modified DNA was purified and recovered according to the specification of the reagent.
3. **The design and synthesis of methylation of specific primers**: According to the whole genomic sequence and the sequence of the promoter region of MMP-9, the CpG island of the MMP-9 gene promoter was searched using the DNA methylation research tool software "MethPrimer" (http://www.urogene.org/methprimer/), and the primer was designed as follows. MMP-9 methylation primer: the sequence of the upstream primer was 5′- GAAGTTCGAAATTAGTTTGGTTAAC-3′, with a length of 25 bp; while the sequence of the downstream primers was 5′-TCCCGAATAACTAATATTATAAACGTA-3′, with a length of 27 bp. MMP-9 non-methylated primer: the sequence of the upstream primer was 5′-AGTTTGAAATTAGTTTGGTTAATGT-3′, with a length of 25 bp; while the sequence of the downstream primer was 5′-CCTCCCAAATAACTAATATTATAAACATA-3′, with a length was 29 bp. The above primers were synthesised by Shanghai Shenggong Biological Engineering Co., Ltd.
4. **Methylation-specific PCR (MSP) amplification**: Thermal cycling parameters were as follows: predenaturation at 95°C for five minutes; denaturation at 94°C for 30 seconds, annealing at 56°C for 30 seconds, extension at 72°C for 30 seconds, for a total of 35 cycles, and finally extended at 72°C for five minutes. PCR products were treated by 1% agarose gel electrophoresis, and the results were observed under a UV lamp.

Determination of MMP-9 concentration in serum samples by ELISA

MMP-9 serum levels were detected using a HUMAN MMP-9 ELISA KIT (Shanghai Langton Biological Technology Co., Ltd.), and experimental operations were strictly performed according to kit instructions.

Statistical methods

All data were processed using SPSS 22.0 software, and measurement data were expressed as mean value ± standard deviation (X ± SD). One-way ANOVA was performed for comparison among groups, and q-test (SNK) was used for pairwise comparisons. Count data were analysed by X2-test. The correlation between parameters was analysed by Spearman's relativity method. $P < 0.05$ was considered statistically significant.

Results

Comparison of general conditions and biochemical indicators

The general conditions and biochemical indicators of the research objects in each group were compared. Results revealed that the difference in gender, age, BMI, SBP, DBP, TG, TC, and LDL-C among the five groups was not statistically significant ($P > 0.05$). The duration of the disease gradually increased with the aggravation of the disease. The duration was longer in the HbA_{1c} DN group than in the simple diabetes group, and there was a difference between the DN group at clinical stage and the early DN group. However, the duration was shorter in the former than that in the early DN group. Furthermore, FPG in the diabetic nephropathy group was higher than in the diabetic group. This was higher in the diabetic nephropathy group at the clinical stage than in the early DN group. Regarding BUN and Cr, these were significantly higher in the DN group at clinical stage than in the simple diabetes group and early diabetes group. In addition, the UACR in the DN group was significantly higher than in the diabetic group. This was significantly higher in the DN group at the clinical stage than in the early DN group. Compared with the simple diabetes group, the GFR of early DN slightly decreased, and this significantly decreased in the DN group at the clinical stage (Table I).

Analysis results of the CpG gene MMP-9 island

The blue part in Figure 1 was the CpG island, which has a total length of 110 bp (367–476).

Statistical analysis of methylation status in the promoter region of the MMP-9 gene

1. Agarose gel electrophoresis results: After MSP amplification, a 108-bp band could be found in some specimens (Fig. 2). If the target bands appear in the specimens amplified both by methylated primers and non-methylated primers, the specimens were determined as semi-methylation status. If the target band only appeared in specimens amplified by methylated primers, the specimens were determined as methylation status, i.e. methylation positive. If the target band only appeared in specimens amplified by non-methylation primers, the specimens were determined as unmethylation state, i.e. methylation negative. The experiment was repeated three times.
2. Statistics of the methylation status of MMP-9 gene promoter: The results indicate (Table II) that, compared with the control group, the positive rate of methylation in the impaired glucose regulation

Table I. *Comparison of each group's general conditions (X ± S)*

Tabela I. *Porównanie ogólnej kondycji pacjentów w obu grupach (± S)*

Variate	Group A	Group B	Group C	Group D	Group E	F/x2	P
Sex (male/female)	13/17	16/14	16/14	16/14	16/14	0.96	0.92
Age (years)	60.37 ± 8.42	61.43 ± 8.63	62.33 ± 6.94	62.67 ± 6.96	63.10 ± 7.05	0.55	0.66
BMI [kg/m²]	24.10 ± 2.63	24.36 ± 2.80	23.77 ± 2.10	23.91 ± 2.48	24.86 ± 2.66	0.86	0.49
SBP [mm Hg]	130.00 ± 7.66	130.83 ± 7.20	132.07 ± 7.06	133.23 ± 7.84	135.17 ± 6.63	2.339	0.58
DBP [mm Hg]	79.20 ± 8.04	81.33 ± 6.69	80.17 ± 7.82	80.17 ± 7.08	83.67 ± 6.29	1.7	0.15
TG [mmol/L]	1.36 ± 0.69	1.46 ± 0.46	1.29 ± 0.42	1.57 ± 0.66	1.47 ± 0.47	1.15	0.34
TC [mmol/L]	4.76 ± 0.71	4.80 ± 0.71	4.59 ± 0.60	4.75 ± 1.34	5.19 ± 0.90	1.91	0.11
LDL-C [mmol/L]	2.81 ± 0.64	2.92 ± 0.82	2.74 ± 0.55	2.92 ± 0.93	3.22 ± 1.01	1.5	0.2
Disease course (years)	0.0 ± 0.0	0.86 ± 0.58	5.96 ± 5.89$^{*\#}$	11.13 ± 5.76$^{*\#\star}$	12.37 ± 6.56$^{*\#\star}$	43.63	< 0.05
HbA_{1c} (%)	5.58 ± 0.25	6.05 ± 0.65	8.12 ± 2.26$^{*\#\star}$	10.02 ± 2.73$^{*\#}$	9.68 ± 2.60$^{*\#}$	31.37	< 0.05
FPG [mmol/L]	4.65 ± 0.62	4.89 ± 0.58	8.49 ± 3.55$^{*\#}$	9.72 ± 2.67$^{*\#}$	10.72 ± 3.64$^{*\#\star}$	27.31	< 0.05
BUN [mmol/L]	5.11 ± 1.10	5.51 ± 1.41	5.50 ± 1.28	6.58 ± 2.02	8.09 ± 4.29$^{*\#\star}$	8.03	< 0.05
Cr [μmol/l]	57.41 ± 9.53	58.12 ± 10.24	63.83 ± 12.29	65.97 ± 17.80	77.77 ± 32.93$^{*\#\star}$	5.78	< 0.05
UACR [mg/mmol]	0.26 ± 0.24	0.84 ± 0.91	0.44 ± 0.53	11.25 ± 7.55$^{*\#\star}$	73.57 ± 28.57$^{*\#\star\star}$	173.6	< 0.05
GFR [ml/min*1.73/m²]	111.97 ± 17.89	114.04 ± 13.94	118.32 ± 18.65*	100.06 ± 20.34*	85.55 ± 40.27$^{*\#\star}$	9.14	< 0.05

P value was corrected for Bonferroni correction; *P < 0.05, compared with group A; #P < 0.05, compared with group B; *P < 0.05, compared with group C; *P < 0.05, compared with group D

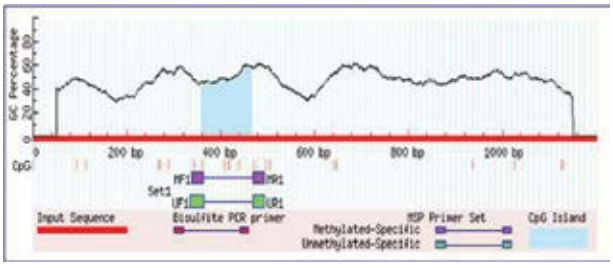

Figure 1. *The blue part is the CpG island, which has a total length of 110 bp (367–476)*

Rycina 1. *Część zaznaczona kolorem niebieskim była wyspą CpG, o łącznej długości 110 pz (367–476)*

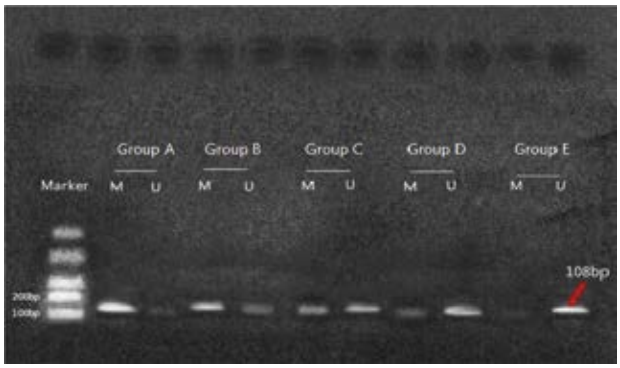

Figure 2. *Agarose gel electrophoresis results: After MSP amplification, a 108-bp band could be found in some specimens*

Rycina 2. *Wyniki elektroforezy w żelu agarozowym: po amplifikacji MSP, w niektórych próbkach znaleziono fragment o długości 108 pz*

Table II. *Statistical analysis of methylation status in the promoter region of the MMP-9 gene*

Tabela II. *Analiza statystyczna statusu metylacji w regionie promotora genu MMP-9*

Group	n	Positive	Positive rate (%)
Group A	30	28	93.33
Group B	30	27	90
Group C	30	14	46.67#
Group D	30	7	23.33#$^{\Delta}$
Group E	30	2	6.67#$^{\Delta\star}$

#P < 0.05,compared with group A; $^{\Delta}$P < 0.05,compared with group C; *P < 0.05, compared with group D

group slightly decreased. However, the difference was not significant, and there was no statistical significance (P > 0.05). Compared with the control group, the positive rates of methylation in the diabetes mellitus group, early DN group, and clinical DN group were significantly decreased, and the difference was statistically significant (P < 0.05). Compared with the diabetes mellitus group, the positive rates of methylation in the early DN group and clinical DN group significantly decreased, and the difference was statistically significant (P < 0.05). Compared with the early DN group, the positive rate of methylation in the clinical DN group significantly decreased, and the difference was statistically significant (P < 0.05).

Detection results of serum MMP-9 levels

Detection results of MMP-9 concentration levels in each group were compared, and these results revealed that concentrations in the impaired glucose regulation group were not significantly changed compared with the control group ($P > 0.05$). However, concentrations in the diabetes mellitus group, DN group and clinical DN group significantly increased ($P < 0.05$). Compared with the diabetes mellitus group, concentrations in the early DN group, and clinical DN group significantly increased, and the difference was statistically significant ($P < 0.05$). In addition, the concentration in the clinical DN group was significantly increased compared with that in the early DN group, and the difference was statistically significant ($P < 0.05$) (Table III).

Table III. *Detection results of serum MMP-9 levels in each group*

Tabela III. *Wyniki wykrywania poziomu MMP-9 w surowicy krwi pacjentów z każdej z grup*

Group	MMP-9 [ng/mL]
Group A	100.87 ± 4.88
Group B	101.93 ± 4.00
Group C	132.67 ± 4.82#
Group D	212.03 ± 5.75#*
Group E	246.27 ± 5.02#*Δ

#P < 0.05, compared with group A; *P < 0.05, compared with group C; ΔP < 0.05, compared with group D

The relationship between the serum expression of MMP-9 and the positive rate of methylation in the promoter region of the MMP-9 gene

The correlation between the serum expression of MMP-9 and the positive rate of methylation in each group was statistically analysed. Results revealed that the expression level of MMP-9 in serum was negatively correlated with the methylation level of the MMP-9 gene promoter region. The analysis results are shown in Figure 3.

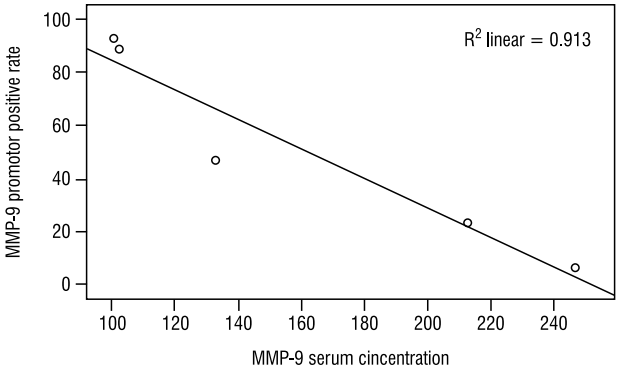

Figure 3. *The results of analysis of the relationship between the serum expression of MMP-9 and the positive rate of methylation in the promoter region of the MMP-9 gene*

Rycina 3. *Wyniki analizy zależności między ekspresją MMP-9 w surowicy krwi a dodatnim tempem metylacji w regionie promotora genu MMP-9*

The correlation analysis of the methylation level of the MMP-9 gene promoter and other variables

Spearman's correlation analysis was performed between the demethylation level of the MMP-9 gene promoter and age, BMI, duration of diabetes mellitus, fasting plasma glucose (FPG), HbA_{1c}, BUN, Cr, UACR, GFR, and serum concentrations of MMP-9 in research objects. Results revealed that the demethylation of the MMP-9 promoter was positively correlated with the serum concentration MMP-9, the duration, FPG, HbA_{1c}, BUN, Cr, and UACR, and was negatively correlated to GFR (Table IV).

Table IV. *The correlation analysis of the methylation level of the MMP-9 gene promoter and other variables*

Tabela IV. *Analiza korelacji poziomu metylacji promotora genu MMP-9 i innych zmiennych*

Variate	Coefficient of association r_S	P value
Age	0.153	0.06
BMI	0.053	0.52
Disease course	0.678*	< 0.05
FPG	0.568*	< 0.05
HbA_{1c}	0.571*	< 0.05
BUN	0.231*	< 0.05
Cr	0.186*	< 0.05
UACR	0.670*	< 0.05
GFR	–0.368*	< 0.05
Serum MMP-9 concentration	0.736*	< 0.05

*when the confidence (double side) is 0.05, the correlation is significant

Discussion

DN is the most common microvascular complication of diabetes mellitus, and it is also the most common cause of ESRD [5]. However, its pathogenesis remains unclear. Genetic susceptibility is an important factor in the occurrence of DN [6], while environmental factors may be involved in the occurrence and development of DN by affecting the changes of epigenetic modifications [7, 8]. DNA methylation modification is one of the most extensively and thoroughly studied epigenetic mechanisms at present. Sapienza C et al. [9] determined the methylation level in DNA extracted from saliva in end-stage DN patients and diabetic patients with no renal complications. These results revealed that there was a significant difference in at least two CpG sites in the two groups of DNAs. This suggests that DNA methylation plays an important role in the pathogenesis of DN and end-stage renal disease.

The role of MMPs in the pathogenesis of diabetes is complex. The study of diabetic mice induced by streptozocin revealed that the decrease in expression levels and activities of MMP-9 and MMP-2 contributed to the accumulation of the renal mesangial matrix [10]. The present experimental results revealed that the serum MMP-9 protein expression levels in the simple diabetes group, early DN group, and clinical DN group were higher than in the normal control group. Serum MMP-9 protein expression level in the early DN group and clinical DN group were higher than in the simple DM group. The MMP-9 expression level in the clinical DN group was higher than in the early DN group. This was consistent with the conclusions on the research on DN carried out by Tashiro K et al. [11], in which the levels of MMP-9 in many albuminuria diabetic patients were significantly higher than that in the normal control group, and this increasing degree was consistent with the clinical staging of the disease. In DN patients, the expression of MMP-9 protein and its catalytic activity increased. However, DN can be assuaged through the suppression of the expression of MMP-9 in kidneys via genetic effects or drug intervention [12]. All these results suggest that MMP-9 may be involved in the occurrence and development of DN.

Experimental results of the MSP analysis in the present study indicate that with the extension of DN duration, the positive rate of methylation significantly decreased ($P > 0.05$). Furthermore, Spearman's correlation analysis revealed that the correlation coefficient rs between the serum MMP-9 concentrations and its demethylation level was 0.736 ($P < 0.05$), suggesting that the demethylation level of the MMP-9 promoter region was positively related to its serum concentration. That is to say, the methylation of the MMP-9 promoter in DN was negatively correlated with serum concentration. Similar conditions were also found in other diseases. Campos et al. found that [13] in periapicalitis, the decreasing extent of methylation of the MMP-9 promoter was consistent with the increasing extent of transcription of MMP-9 mRNA. Roach et al. found that [4] the high expression of MMP-9 was associated with the low methylation level of the MMP-9 gene. In patients with non-small cell lung cancer, the average methylation density of the MMP-9 promoter was significantly lower than that in the healthy group, which revealed a significantly lower methylation status [14]. The experiment in vitro confirmed that the methylation status of the MMP-9 gene promoter played an inhibition role in transcriptional activity. It was observed in lymphocyte cultivation that the methylation status of the MMP-9 gene promoter was negatively correlated with MMP-9 mRNA and protein expression levels. When lymphocytes were treated by the DNA methylation inhibitor, the methylation status of the MMP-9 gene promoter could be reduced, and the mRNA and protein expression of MMP-9 could be promoted [15].

In the present experiment, the positive rate of methylation of the MMP-9 promoter in the impaired glucose regulation group was slightly lower than that in the control group. However, the difference was not significant ($P > 0.05$). This may be related to the duration and severity of the disease, the duration of patients in the impaired glucose regulation group was relatively short, the regulating ability and metabolic function were acceptable, and the factors affecting the activity of MMP-9 transcription may be fever. Spearman's correlation analysis results revealed that the demethylation of the MMP-9 promoter region was positively related to the duration of the disease, fasting plasma glucose (FPG), and HbA_{1c}, which suggests that longer disease duration and poor blood glucose control resulted in a greater impact of MMP-9 transcriptional levels and a greater expression of MMP-9, thus affecting the progression of DN. Roach [9] et al. found in the study on the cartilage of osteoarthritis that the expression of MMP-9 increased compared with the normal control group, and this was related to the low methylation level of the MMP-9 gene, which was consistent with the results of the present experiment.

In the present experiment, we attempted to eliminate the controllable factors for comparative analysis. In fact, there are many deficiencies in this study: the sample numbers were fewer, and DN is usually diagnosed by renal biopsy, However, it is difficult to practice in clinical trials. The experiments in vivo were affected by factors such as emotion, real-time blood glucose variations, and other. Furthermore, the MMP-9 mRNA levels were not detected, and no sequencing analysis was performed after MSP amplification. In future research, the methylation status of local kidney tissue can be selected to compare with that of the peripheral blood in animal experiments, the in vitro cell experiment can also be performed to exclude interference factors, and the sample size can be increased in order to study and validate the aberrant methylation of MMP-9 gene promoter in DN, as well as to elaborate the role of the methylation of the MMP-9 gene promoter in the occurrence and development of DN more comprehensively, so as to provide a new basis for the early prevention and treatment of DN.

Conclusions

The demethylation of the MMP-9 gene promoter may be involved in the occurrence and development of DN by regulating the expression of MMP-9 protein in serum.

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